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Detection of a Nuclear Antigen 2 (EBNA2)-Variant Epstein-Barr Virus Strain in Two Siblings With Fatal Lymphoproliferative Disease

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An EBV type 1 variant strain was detected in two Turkish siblings (boy and girl), who both suffered and died from similar progressive Epstein-Barr virus (EBV)-associated lymphoproliferative disease. Molecular characterisation of this EBV isolate revealed a 51bp-deletion and six nucleotide changes within the Epstein-Barr nuclear antigen 2 (EBNA2). Both isolates contained EBV type 2 sequences in the Epstein-Barr virus-encoded small RNAs (EBER), which are 40 kb proximal to EBNA2. Sequencing of the EBV isolates in a region of Epstein-Barr nuclear antigen 3 (EBNA3a), which is 40 kb distal to EBNA2, revealed the normal EBV type 1 sequence of laboratory strain B95-8. This EBV isolate may represent a distinct wild type EBV strain with altered biological properties. It is suggested that this EBNA2-variant strain may be responsible at least in part for the severe clinical course in both affected children.

KEY WORDS: EBNA2 sequence, EBNA3a sequence, EBER genotype, PCR

INTRODUCTION

Epstein-Barr virus (EBV), a ubiquitous human B lymphotropic virus, is the causative agent of acute infectious mononucleosis, usually a self-limiting polyclonal lymphoproliferative disease with good prognosis. In subjects with inherited or acquired immunodeficiency disorders, EBV infection may lead to life-threatening lymphoproliferative disorders and lymphoma [Hano et al., 1985]. Fatal primary EBV infections in children and adults are rare events. A sporadic form of fatal infectious mononucleosis (FIM) occurs in 1 in 3,000 cases of infectious mononucleosis [Purtilo et al., 1992]. The median age of these patients is 13 years [Mroczek et al., 1987]. An inherited form of FIM is found in 46% of boys who carry the defective gene of X-linked lymphoproliferative disease (XLP), an inherited immunodeficiency with a strong association with EBV infections [Purtilo et al., 1975; Schuster et al., 1993]. The mean age at onset is 2.5 years. In a similar hereditary syndrome with susceptibility to severe EBV infection but with a non-X-linked, probably autosomal form of inheritance, girls also may be affected by fatal EBV infection [Fleisher et al., 1982; Inaba et al., 1989; Purtilo et al., 1981]. Presenting signs and symptoms of all forms of FIM are similar and include high fever, sore throat, cervical or generalised lymphadenopathy, hepatosplenomegaly, malaise, and a maculopapular skin rash. With disease progression, pancytopenia, bone marrow failure, and encephalopathy frequently develop. Hepatic involvement may be complicated by coagulopathy. Fulminant hepatitis and/or multiorgan failure is often the cause of death.

The aetiology of FIM is still not clear. In addition to genetic factors that seem to play a major role in XLP or other EBV-associated hereditary syndromes, certain immunological and possibly also viral factors may determine clinical course and outcome of EBV infections.

As far as viral factors are concerned, the Epstein-Barr nuclear antigen (EBNA2) is of particular interest. This protein is essential for the transformation/immortalisation process of B lymphocytes by EBV [Cohen et al., 1991]; it seems to be one critical determinant for EBV-induced lymphoma tumour growth in immunodeficient SCID mice [Cohen et al., 1992]. It has been shown that mutations and deletions within the EBNA2 gene have a direct influence on the efficacy of B cell growth transformation *in vitro* and *in vivo* in SCID mice [Cohen et al., 1991, 1992]. EBNA2-deleted mutant strains have been identified in oral hairy leukoplakia (HLP) lesions of HIV-infected patients [Sixbey et al., 1991; Walling et al., 1994]. This probably reflects a positive selection for EBNA2- defective variants as part of the viral survival strategy.

The present report describes two siblings (girl and boy) both suffering from similar progressive fatal EBV-associated lymphoproliferative disease. In both children, an EBV type 1 variant strain harbouring a 51-bp deletion and six nucleotide changes within the EBNA2

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gene was identified. In addition, there was no cytotoxic T-cell response against autologous EBV-transformed B cells and K562 target cells in the younger boy [Schuster et al., 1990]. It is suggested therefore that both immunological and viral factors may have contributed substantially to the severe clinical course.

MATERIALS AND METHODS

Patients

Child 1. This female child was born in Germany to healthy Turkish parents in May 1980. Four older siblings were healthy, whereas four other siblings had died of unknown cause in Turkey. At the age of 15 months, the previously healthy girl was admitted to the hospital because of severe infectious mononucleosis with high fever, pharyngitis, generalised lymphadenopathy, hepatosplenomegaly, and encephalopathy. Hepatic enzymes were increased. The cerebrospinal fluid contained 53 lymphoid cells/μl. The serum IgG level was 1,974 mg/dl (normal range for age: 490–690 mg/dl), IgM was 430 mg/dl (normal range for age: 65–105 mg/dl), and IgA level was 180 mg/dl (normal range for age 15–85 mg/dl). The hemoglobin level was 100 g/L, the white blood cell count was 13.3×10^9/L, with 15% neutrophils and 60% lymphocytes. The thrombocyte count was 175×10^9/L. The proportions of circulating T and B lymphocytes were normal. However, the fraction of $CD8^+$ T cells was high (62%), whereas that of $CD4^+$ T cells was low (6.8%). The CD4/CD8 ratio was 0.11. Antibody titres (IgG) against EBV early antigen (EA) and viral capsid antigen (VCA), as determined by indirect immunofluorescence, were 1:256 and 1:512, respectively. The antibody titre against Epstein-Barr nuclear antigen (EBNA) was 1:16 by anticomplement immunofluorescence, and there were faint, but definite bands against EBNA1 as revealed by immunoblot testing. Neither IgM and IgA antibodies against VCA nor IgG antibodies against EBNA2 were found. Large amounts of EBV DNA were detected in a lymph node biopsy specimen by Southern blot hybridization. HLA typing was not done.

The girl's clinical condition deteriorated continuously. Generalised lymphadenopathy and hepatosplenomegaly increased further: the child became lethargic and developed a left-sided Bell's palsy 20 weeks after admission. Five weeks later, the patient died from severe gastrointestinal bleeding and multiorgan failure. Autopsy was refused by the parents.

Child 2. This boy, a younger brother of the first child, was born in Germany in September 1983. He had a history of recurring abscesses on his left thigh by 15 months of age. There was no evidence of a phagocytic disorder. At the age of 30 months, he was admitted to the Children's hospital of Würzburg University because of severe infectious mononucleosis with fever, hepatosplenomegaly, generalised lymphadenopathy, and acquired hypogammaglobulinemia. Serum IgG was 319 mg/dl (normal range for age: 520–1370 mg/dl), IgM was 39 mg/dl (normal range for age: 40–206 mg/dl), and the IgA level was 19 mg/dl (normal range for age: 32–180 mg/dl). Serum IgG antibody titres against EA, VCA, and EBNA were 1:64, 1:512, and 1:64, respectively. Antibodies against EBNA1 and EBNA2 were found by either immunoblot or EIA. IgM and IgA antibodies against VCA were absent. EBV DNA could be detected in a lymph node biopsy and a bone marrow aspirate [Schuster et al., 1990]. Cells from a lymph node biopsy showed monoclonal rearrangements of immunoglobulin heavy chain genes (J_H) but no clonal rearrangements of T-cell receptor β-chain genes (TCR_β) or immunoglobulin kappa chain genes (C_k).

The fraction of $CD8^+$ T cells was high (34%). The CD4/CD8 ratio was slightly decreased (1.1). Activated $CD8^+$ T cells showed no cytolytic activity *in vitro* against autologous EBV- transformed B cells and K562 target cells [Schuster et al., 1990]. There were, however, exceptionally high serum levels of neopterin (147 nmol/L; normal range for age 3.5–13.5 nmol/L) and interferon gamma (16,637 U/L; normal up to 100 U/L) [Schuster et al., 1992]. These results pointed to a maximally stimulated, but ineffective immune response of the host. The boy revealed the following HLA type: A25, A29, B7, B51, (Bw4, Bw6), Cw7, DR11(5) [HLA allele DRB1*1101] and DR52 [HLA allele DRB3*0202].

The clinical condition of the patient worsened in spite of treatment with acyclovir, steroids, and cyclophosphamide. The boy died 4 months after admission. Permission for autopsy was not given by the parents.

Other EBV-seropositive family members were not affected by fatal EBV-lymphoproliferative disease. In this family molecular genetic analysis with polymorphic DNA markers from Xq24-q26 excluded cosegregation with the X-linked lymphoproliferative disease (XLP) gene locus (data not shown).

Polymerase Chain Reaction (PCR) and Direct Sequencing of PCR Products

Genomic DNA was extracted from a diagnostic lymph node biopsy (Child 1) and a bone marrow aspirate (Child 2) according to standard procedures. DNA samples were amplified by PCR using the following primers specific for sequences within the EBV type 1 nuclear antigen 2 (EBNA2): 5′-TCTTGATAGGGATCCGCTAGGATA-3′ (nucleotide positions 1842–1865) and 5′-ACCGTGGTTCTGGACTATCTGGATC-3′ (nucleotide positions 2338–2314) [Dambaugh et al., 1984; Jilg et al., 1990]. For amplification of a part of the coding region of EBV nuclear antigen 3a (EBNA3a), which is 40 kb distal to the EBNA2 gene, the following primers were used: 5′-GAAACCAAGACCAGAGGTCC-3′ (forward) and 5′-CCCAGGGCCGGACAATAGG-3′ (reverse) [Sample et al., 1990]. PCR conditions have been reported elsewhere [Jilg et al., 1990; Sample et al., 1990]. Double- stranded PCR-products were purified with PrimeErase Quick™ Push Columns (Stratagene, Heidelberg, Germany) and directly cycle-sequenced by the dideoxy- termination method [Sanger et al., 1977] using ^{35}S-dATP and the Exo(-)Pfu Cyclist™ DNA Sequencing Kit (Stratagene) according to the manufacturer's protocol. Samples were electrophoresed

TABLE I. Molecular Characterisation of an EBNA2 Mutant EBV Strain Isolated From Two Siblings With Fatal Lymphoproliferative Disease

	EBNA2 nucleotide changes (in comparison to EBVtype 1 strain B95-8)							EBV genotype in the EBER region
Nucleotide[a]	1,993/4	2,001	2,060	2,094	2,116	2,122–2,172	2,191	
B95-8 (EBV type 1)	AG	C	A	G	A		C	EBV type 1
	Arg	*Val*	*Gln*	*Met*	*Thr*		*Leu*	
Child 1 and 2	GT	A	G	T	T	51bp-deletion[b]	T	EBV type 2
	Val	*Val*	*Arg*	*Ile*	*Ser*		*Phe*	
AG876 (EBV type 2)	AC	A	C	C	A		C	EBV type 2
	Thr	*Glu*	*Ala*	*Ala*	*Thr*		*Pro*	

[a]Numbering of nucleotides is rendered according to Dambaugh et al. [1984].
[b]EBV isolates from both children exhibited an identical 51bp-deletion (nucleotide positions 2,122 through 2,172); CTG CCA CCT GCA ACA CTA ACG GTG CCA CCA AGG CCT ACC CGT CCT ACC ACT.

on a 6% polyacrylamide/8.3 M urea sequencing gel (Roth, Karlsruhe, Germany), dried, and exposed to Kodak XAR-5 films.

PCR-SSCP Analysis

For genotyping in the EBER region (EBV-encoded small RNAs), which is 40 kb proximal to the EBNA2 gene, we used single-strand conformation polymorphism (SSCP) analysis, which can detect genotype-specific point mutations within this region by characteristic shifts in mobility due to conformational changes of the DNA sequences. PCR conditions have been published elsewhere [Lin et al., 1993]. The following EBER-specific primers were used :5′-GTGGTCCGCATGTTTTGATC-3′ (nucleotide positions 6,780–6,800) and 5′- GCAACGGCTGTCCTGTTTGA-3′ (nucleotide positions 6,969–6,950) [Lin et al., 1993]. PCR products were radiolabeled by inclusion of 2 μCi [alpha-^{32}P]-dCTP (3000 Ci/mmol) in the PCR. The amplified 190 bp products were heat-denatured and analysed in a nondenaturing 5% polyacrylamide gel (Roth) containing 5% glycerol in 0,5xTBE buffer at room temperature for 7 h at 10 W. The gel was dried and exposed to Kodak XAR-5 film for up to 4 days.

RESULTS

EBNA2-specific PCR-products from DNA of a lymph node biopsy (Child 1) and a bone marrow aspirate (Child 2) were both smaller than those from other wild-type EBV type 1 isolates or with EBV type 1 prototype strains B95-8 and Raji (not shown). Sequencing of these shorter PCR-products (Fig. 1a,b) revealed an identical 51-bp deletion from nucleotide position 2,122–2,172, which accounts for a loss of 17 amino acids (Leu-Pro-Pro-Ala-Thr-Leu-Thr-Val-Pro-Pro-Arg-Pro-Thr-Arg-Pro-Thr-Thr). Moreover, identical nucleotide changes were found in both children at positions 1,993 (A → G), 1,994 (G → T), 2,001 (C → A), 2,060 (A → G), 2,094 (G → T), 2,116 (A → T), and 2,191 (C → T), leading to amino acid substitutions at position 166 (Arg → Val), 188 (Gln → Arg), 199 (Met → Ile), 207 (Thr → Ser), and 232 (Leu → Phe) (Table I).

PCR amplification of a section of the EBNA3a coding region generated the normal-size 276-bp DNA fragment in both the children's EBV isolates as also found for EBV type 1 strain B95-8 (not shown). Direct sequencing of these PCR-products revealed the unchanged standard nucleotide sequence of the B95-8 strain (not shown).

PCR-SSCP analysis of the children's EBV isolates in the EBER region revealed an EBV type 2-specific band pattern in both cases (Fig. 2).

An EBV-transformed B cell line (B-LCL) was established from the second child by infection of the patient's peripheral blood lymphocytes with EBV type 1 laboratory strain B95-8. In this B-LCL, both the shortened EBNA2-specific PCR-product of the mutant EBV strain as well as the full-size PCR fragment of EBV strain B95-8 were found (not shown). B95-8-transformed B-LCL established from peripheral blood lymphocytes of seven healthy EBV-seropositive family members (both parents, two sisters, three brothers) contained only EBV laboratory strain B95-8 and *not* the EBNA2 mutant strain (not shown). Furthermore, in cryopreserved peripheral blood mononuclear cells (PBMC) of one older sister, EBV type 1 with EBNA2 sequences of laboratory strain B95-8 was detected. Spontaneously outgrowing B-LCL could not be established in any of the healthy seropositive family members (mother, father, one sister, two brothers). It was therefore not possible to determine the source of the mutant virus.

DISCUSSION

In the family reported here, one girl and one of her younger brothers suffered and died from severe EBV-associated lymphoproliferative disease, suggesting a non-X-linked inherited disorder. There are only very few reports on other families where hereditary FIM have affected both males and females [Fleisher et al., 1982; Inaba et al., 1989; Purtilo et al., 1981]. The primary defect in this rare hereditary disorder is not known. Common findings in affected subjects are a decreased NK cell activity and a history of recurrent bacterial infections [Fleisher et al., 1982; Inaba et al., 1989]. However, since NK cell activity has been shown to reappear in one of these patients who survived severe EBV infection [Starr et al., 1990], the significance of this finding is not clear.

One of the two children reported here also had a history of recurring bacterial infections and exhibited a

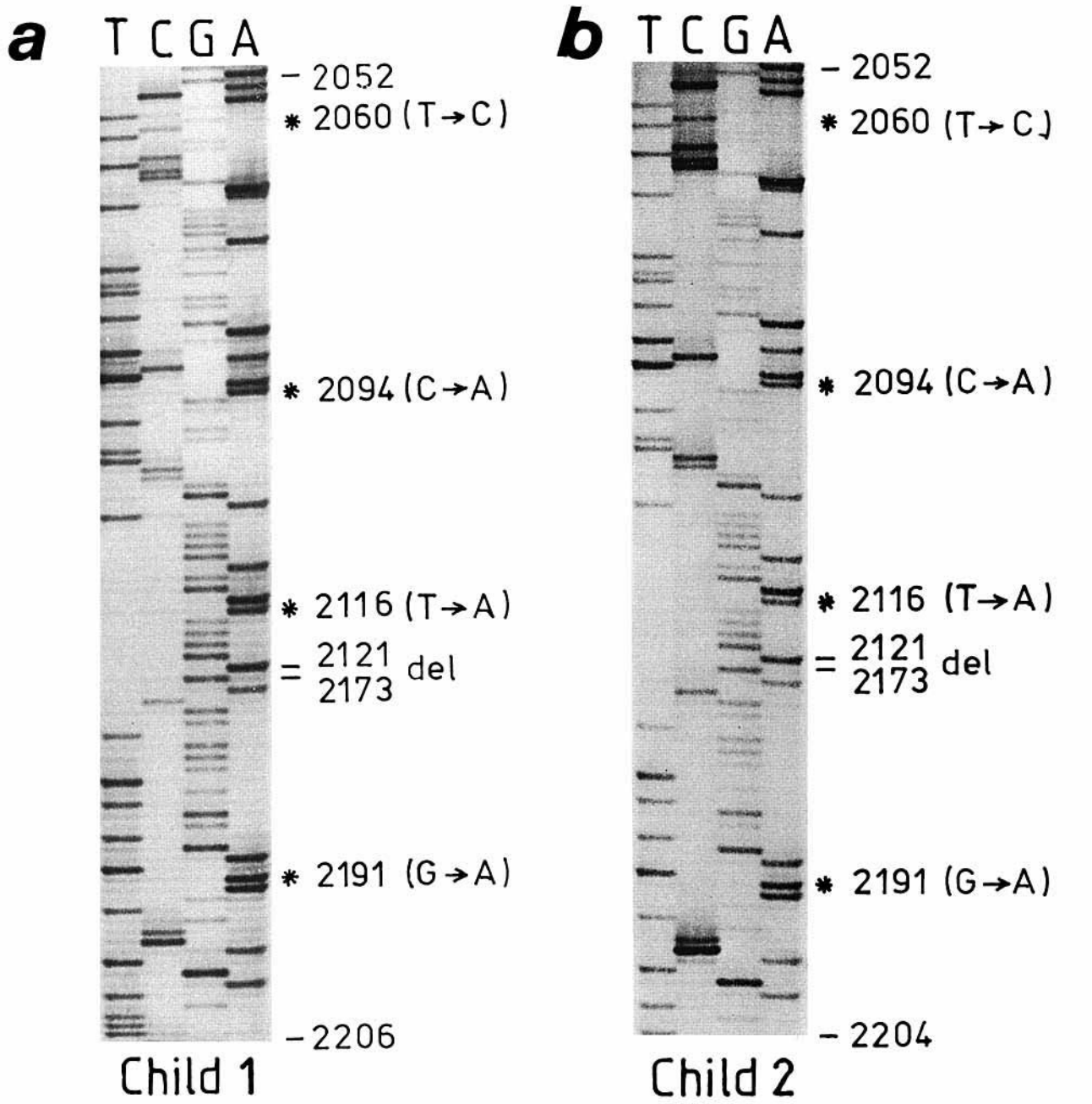

Fig. 1. (**a,b**) Nucleotide sequencing pattern of the EBNA2 region (*noncoding, complementary* strand from position 2,052 to 2,204/2,206 is shown) from the female sibling (Child 1, 1a) and her younger brother (Child 2, 1b) showing in both cases a 51-bp deletion (del) from position 2,122 through 2,172 and nucleotide changes (*) at positions 2,060, 2,094, 2,116, and 2,191. Numbering of nucleotides is rendered according to Dambaugh et al. [1984].

deficient NK cell activity [Schuster et al., 1990]. Moreover, this child showed a deficient lymphocyte-mediated EBV-specific cytotoxicity as well as acquired hypogammaglobulinemia. This may suggest that a selective immune deficiency was primarily responsible for the inability to control EBV infection in affected family members.

In two families FIM occurred after the age of 14 years [Fleisher et al., 1982; Inaba et al., 1989]. In contrast, both children reported here, as well as two siblings from a Swedish family (girl and boy) with a probable similar hereditary disorder [Purtilo et al., 1981], had succumbed to primary EBV infection before the age of 5 years, suggesting genetic heterogeneity between affected families. This earlier clinical manifestation is similar to X-linked lymphoproliferative disease (XLP)

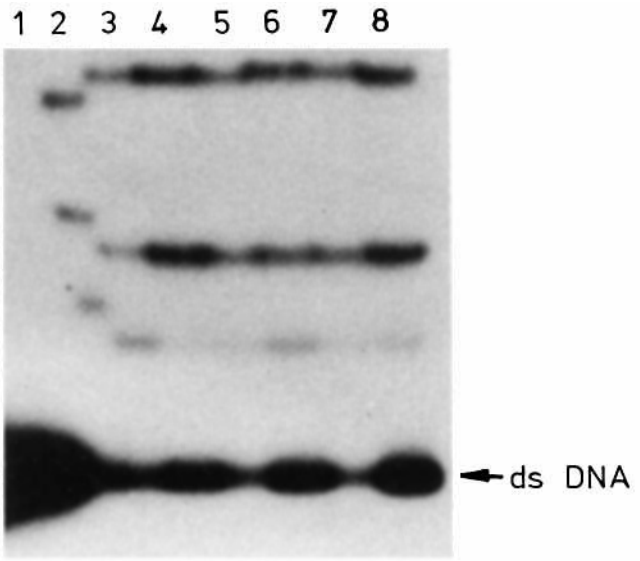

Fig. 2. PCR-SSCP analysis of EBER region. Genotype-specific point mutations were visualised as shifts in mobility. The denatured (single- stranded) DNA resolved into two main bands: The slowest moving band represents the antisense DNA strand, the second band is due to the sense strand [Lin et al., 1993]. The fainter third band is possibly caused by a faster migrating complex of partially denatured sense and antisense strands. The nondenatured double-stranded DNA (dsDNA) migrated as a single band with the fastest mobility. **Lane 1:** nondenatured double-stranded DNA (control, B95-8), **Lane 2:** B95-8 (EBV type 1 prototype strain), **Lane 3:** Jijoye (EBV type 2 prototype strain), **Lane 4,5:** EBV isolates from child 1 and 2, respectively, **Lanes 6–8:** EBV isolates from three children with X-linked lymphoproliferative disease (XLP).

where the mean age at onset of primary EBV infection is 2.5 years [Seemayer et al., 1993].

Both children reported here exhibited persistently elevated serum antibody levels against EBV replicating antigens (EA). This may point to unregulated replication of EBV suggesting to be a central mechanism of the fatal lymphoproliferative course of disease in both cases. A similar serological finding is also seen regularly in children suffering from severe chronic active EBV infection [Okano et al., 1991].

In addition, the girl had an unusual serological response to the EBNA complex, since she developed low antibody titres to EBNA1 but no antibodies against EBNA2. Normally, 93% of patients with infectious mononucleosis produce antibodies against EBNA2 within 2–3 months after onset of disease [Henle et al., 1987]. In addition, within the first year after onset of mononucleosis, serum levels of antibodies to EBNA2 normally exceed the titres of EBNA1 antibodies [Henle et al., 1987]. In patients with chronic active EBV infection, anti-EBNA1/anti-EBNA2 ratios below 1.0 may even persist for years [Henle et al., 1987; Seigneurin et al., 1987]. The reason for the absent serological response against EBNA2 in the girl remains unclear. Possible explanations could be that: (1) this child harboured a variant EBV strain with a mutant, less immunogenic EBNA2 protein, (2) the patient's EBV strain did not express EBNA2 *in vivo* (as seen in Burkitt lymphoma), or (3) that in a selective manner the girl's immune system did not react to EBNA2. However, it should be noted that EBNA2 antibodies were detected in the serum of the younger brother infected with the same EBV strain.

In both children, but not in other healthy family members, a mutant EBV type 1 variant strain was identified with an identical deletion within the EBNA2 gene. Since neither EBV type 2 nor other additional EBV type 1 strains were found in lymphatic tissues from either child, it is suggested that in addition to genetic and immunological factors, this EBNA2-variant EBV substrain substantially contributed to the severe course of the disease. Other investigators have found transforming-incompetent defective EBV strains in B cells of some patients with chronic active EBV infection [Alfieri et al., 1987; Schooley et al., 1986]. It is not known if these EBV isolates carried an EBNA2 mutation as described here. An EBV type 1 variant strain with a similar 51bp-deletion within EBNA2 to that described in the two children has been recently found in two New Guinea Burkitt lymphoma cell lines (L3, L8) [Aitken et al., 1994]. It is not clear so far if these EBNA2 mutant strains are associated predominantly with malignant lymphoma or severe lymphoproliferative disorders.

It has been shown for other viruses (polio, measles, rubella, hepatitis B, HIV, and others) that mutations/deletions in the viral genome may increase or decrease the virulence of the infectious agent [Chantler et al., 1993; Guillot et al., 1994; Hasegawa et al., 1994; Huang et al., 1995; Kirchhoff et al., 1995; Ubol et al., 1994]. To what extent certain EBV mutants may also influence directly the clinical course of the disease is not known at present. It has been suggested recently, that some EBV-associated malignant tumours such as Hodgkin disease and nasopharyngeal carcinoma harbouring EBV strains with certain deletions in the latent membrane antigen 1 (LMP1), may exhibit a more aggressive behaviour [Chen et al., 1992; Knecht et al., 1993]. However, similar LMP1-mutations also have been found in isolates from subjects with uncomplicated infectious mononucleosis [Sandvej et al., 1994].

EBNA2, one of the most important latent genes of EBV, plays a crucial role in the transformation/immortalisation process of B cells infected with EBV. It could be demonstrated, at least *in vitro*, that certain mutations within the EBNA2 gene can lead to a different transforming activity, which also may affect the virulence of the virus [Cohen et al., 1991, 1992]. The EBNA2-variant strain detected in the two children may be still able to transform B lymphocytes and to transactivate other viral genes, since the reported deletion (amino acids 209–225) encompasses a region within the EBNA2 gene that seems not to be essential for transformation [Cohen et al., 1991; Ling et al., 1993].

EBNA2-defective EBV substrains have been characterised in lytically infected epithelial lesions of oral hairy leukoplakia (OHL) [Walling et al., 1994]. It has been shown that these EBNA2 deletion variants either

evolved by increased replication frequency (from an originally intact EBNA2 gene) or appeared to be "stable" transmissible substrains that directly caused the OHL lesion [Walling et al., 1994]. In the case of the children reported here, both EBV isolates revealed identical EBNA2 mutations and EBER genotypes, probably representing a stable transmissible EBV substrain. Studies in animals infected with EBV variant strains have to show whether certain mutations in EBNA2, LMP1, or other viral antigens will increase or decrease the virulence of EBV.

EBNA2 is one of the EBV target antigens recognized by HLA class I-restricted cytotoxic T cells [Lee, 1994]. However, the EBNA2 deletion described here does not contain either any of the immunodominant T-cell epitopes characterised so far [Khanna et al., 1992; Lee, 1994; Thomson et al., 1995] nor any of the known MHC class I and II peptide motifs with regard to the boy's HLA type [Rammensee et al., 1995]. It has been suggested that EBNA2-deleted EBV strains [Walling et al., 1994] or other EBV variant strains with a single HLA-restricted viral epitope loss [de Campos-Lima et al., 1993] may escape selectively from the host's immunosurveillance. There is, however, increasing evidence that generation of biologically relevant CTL escape virus variants *in vivo* requires selection of mutations in more than one and likely in all the viral CTL epitopes [Lewicki et al., 1995].

Further studies are required to clarify the complex interactions between genetic, immunological, and viral factors in fatal mononucleosis and to determine whether mutant EBV strains with deletions/mutations in EBNA2 or other viral antigens may play a significant role in this severe clinical manifestation. These studies may eventually help to develop new therapeutic approaches in the future such as polyepitope CTL vaccines [Thomson et al., 1995] that might be able to induce suppression of mutant EBV strains.

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REFERENCES

Aitken C, Sengupta SK, Aedes C, Moss DJ, Sculley TB (1994): Heterogeneity within the Epstein-Barr virus nuclear antigen 2 gene in different strains of Epstein-Barr virus. Journal of General Virology 75:95–100.

Alfieri C, Joncas JH (1987): Biomolecular analysis of a defective nontransforming Epstein-Barr virus (EBV) from a patient with chronic active EBV infection. Journal of Virology 61:3306–3309.

Chantler JK, Lund KD, Miki NP, Berkowitz CA, Tai G (1993): Characterization of rubella strain differences associated with attenuation. Intervirology 36:225–236.

Chen ML, Tsai CN, Liang CL, Shu CH, Hang CR (1992): Cloning and characterization of the latent membrane protein (LMP) of a specific Epstein- Barr virus variant derived from the nasopharyngeal carcinoma in the Taiwanese population. Oncogene 7:2131–2140.

Cohen JI, Picchio GR, Mosier DE (1992): Epstein-Barr virus nuclear protein 2 is a critical determinant for tumor growth in SCID mice and for transformation in vitro. Journal of Virology 66:7555–7559.

Cohen JI, Wang F, Kieff E (1991): Epstein-Barr virus nuclear protein 2 mutations define essential domains for transformation and transactivation. Journal of Virology 65:2545–2554.

Dambaugh T, Hennessy K, Chamnankit L, Kieff E (1984): U2 region of Epstein-Barr virus DNA may encode Epstein-Barr nuclear antigen 2. Proceedings of the National Academy of Sciences, USA 81:7632–7636.

de Campos-Lima PO, Gavioli R, Zhang QJ, Wallace LE, Dolcetti R, Rowe M, Rickinson AB, Masucci MG (1993); HLA-A11 epitope loss isolates of Epstein-Barr virus from a highly A11 + population. Science 260:98–100.

Fleisher G, Starr S, Koven N, Kamiya H, Douglas SD, Henle W (1982): A non-X-linked syndrome with susceptibility to severe Epstein-Barr virus infection. Journal of Pediatrics 100:727–730.

Guillot S, Otelea D, Delpeyroux F, Crainic R (1994): Point mutations involved in the attenuation/neurovirulence alternation in type 1 and 2 oral polio vaccine strains detected by site-specific polymerase chain reaction. Vaccine 12:503–507.

Hanto DW, Frizzera G, Gajl-Peczalska KJ, Simmons RL (1985): Epstein-Barr virus, immunodeficiency, and B cell lymphoproliferation. Transplantation 39:461–472.

Hasegawa K, Huang J, Rogers SA, Blum HE, Liang TJ (1994): Enhanced replication of a hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. Journal of Virology 68:1651–1659.

Henle W, Henle G, Andersson J, Ernberg I, Klein G, Horwitz CA, Marklund G, Rymo L, Wellinder C, Straus SE (1987): Antibody responses to Epstein-Barr virus-determined nuclear antigen (EBNA)-1 and EBNA-2 in acute and chronic Epstein-Barr virus infection. Proceedings of the National Academy of Sciences, USA 84:570–574.

Huang Y, Zhang L, Ho DD (1995): Characterization of nef sequences in long-term survivors of human immunodeficiency virus type 1 infection. Journal of Virology 69:93–100.

Inaba T, Hanada R, Yaginuma A, Hayashi Y, Kamamoto K, Hukada H (1989): Fatal infectious mononucleosis with staphylococcal pyoderma in a girl with hereditary immunological dysregulations. European Journal of Pediatrics 149:177–178.

Jilg W, Sieger E, Alliger P, Wolf H (1990): Identification of type A and B isolates of Epstein-Barr virus by polymerase chain reaction. Journal of Virological Methods 30:319–322.

Khanna R, Burrows SR, Kurilla MG, Jacob CA, Misko IS, Sculley TB, Kieff E, Moss DJ (1992): Localisation of Epstein-Barr virus cytotoxic T cell epitopes using recombinant vaccinia: implications for vaccine development. Journal of Experimental Medicine 176:169–178.

Kirchhoff F, Greenough TG, Brettler DB, Sullivan JL, Desrosiers RC (1995): Absence of intact nef sequences in a long-term survivor with nonprogressive HIV-1 infection. New England Journal of Medicine 332:228–232.

Knecht H, Bachmann E, Brousset P, Sandvej K, Nadal D, Bachmann F, Odermatt BF, Delsol G, Pallesen G (1993): Deletions within the LMP1 oncogene of Epstein-Barr virus are clustered in Hodgkin's disease and identical to those observed in nasopharyngeal carcinoma. Blood 10:2937–2942.

Lee SP (1994): Control mechanisms of Epstein-Barr virus persistence. Seminars in Virology 5:281–288.

Lewicki H, Tishon A, Borrow P, Evans CF, Gairin JE, Hahn KM, Jewell DA, Wilson IA, Oldstone MB (1995): CTL escape viral variants. I.Generation and molecular characterization. Virology 210: 29–40.

Lin JC, Lin SC, De BK, Chan WP, Evatt BL (1993): Precision of genotyping of Epstein-Barr virus by polymerase chain reaction using three gene loci (EBNA-2, EBNA-3C, and EBER): predominance of type A virus associated with Hodgkin's disease. Blood 81:3372–3381.

Ling PD, Ryon JJ, Hayward SD (1993): EBNA-2 of herpesvirus Papio diverges significantly from the type A and type B EBNA-2 proteins of Epstein-Barr virus but retains an efficient transactivation domain with a conserved hydrophobic motif. Journal of Virology 67:2990–3003.

Mroczek EC, Weisenburger DD, Grierson HL, Markin R, Purtilo DT (1987): Fatal infectious mononucleosis and virus-associated hemophagocytic syndrome. Archives of Pathology and Laboratory Medicine 111:530–535.

Okano M, Matsumoto S, Osato T, Sakiyama Y, Thiele GM, Purtilo DT (1991): Severe chronic active Epstein-Barr virus infection syndrome. Clinical Microbiology Reviews 4:129–135.

Purtilo DT, Cassel CK, Yang JP, Harper R (1975): X-linked recessive progressive combined variable immunodeficiency (Duncan's disease). Lancet I:935–940.

Purtilo DT, Sakamoto K, Saemundsen AK, Sullivan JL, Synnerholm A-C, Anvret M, Pritchard J, Sloper C, Sieff C, Pincott J, Pachman L, Rich K, Cruzi F, Cornet JA, Collins R, Barnes N, Knight J, Sandstedt B, Klein G (1981): Documentation of Epstein-Barr virus infection in immunodeficient patients with life-threatening lymphoproliferative diseases by clinical, virological, and immunopathological studies. Cancer Research 41:4226–4236.

Purtilo DT, Strobach RS, Okano M, Davis JR (1992): Biology of disease: Epstein-Barr virus-associated lymphoproliferative disorders. Laboratory Investigation 67:5–23.

Rammensee HG, Friede T, Stefanovic S (1995): MHC ligands and peptide motifs: first listing. Immunogenetics 41:178–228.

Sample J, Young L, Martin B, Chatman T, Kieff E, Rickinson A, Kieff E (1990): Epstein-Barr virus types 1 and 2 differ in their EBNA-3A, EBNA-3B, and EBNA-3C genes. Journal of Virology 64:4084–4092.

Sandvej K, Peh S-C, Andresen BS, Pallesen G (1994): Identification of potential hot spots in the carboxy-terminal part of the Epstein-Barr virus (EBV) BNLF-1 gene in both malignant and benign EBV-associated diseases: High frequency of a 30-bp deletion in Malaysian and Danish peripheral T-cell lymphomas. Blood 84: 4053–4060.

Sanger F, Nicklen S, Coulson A (1977): DNA sequencing with chain terminating inhibitors. Proceedings of the National Academy of Sciences USA 74:5463–5467.

Schooley RT, Carey RW, Miller G, Henle W, Eastman R, Mark EJ, Kenyon K, Wheeler EO, Rubin RH (1986): Chronic Epstein-Barr virus infection associated with fever and interstitial pneumonitis. Annals of Internal Medicine 104:636–643.

Schuster V, Kreth HW, Müller-Hermelink HK, Huppertz HI, Feller AC, Neumann-Haefelin D, Wiegand H, Müller-Lantzsch N (1990): Epstein-Barr virus infection rapidly progressing to monoclonal lymphoproliferative disease in a child with selective immunodeficiency. European Journal of Pediatrics 150:48–53.

Schuster V, Reibnegger G, Wachter H (1992): Neopterin and endogenous interferon gamma in serum of patients with Epstein-Barr virus-associated lymphoproliferative diseases. Pteridines 2:189–191.

Schuster V, Kreß W, Friedrich W, Grimm T, Kreth HW (1993): X-linked lymphoproliferative disease: Detection of a paternally inherited mutation in a German family using haplotype analysis. American Journal of Diseases of Children 147:1303–1305.

Seemayer TA, Grierson H, Pirruccello SJ, Gross TG, Weisenburger DD, Davis J, Spiegel K, Brichacek B, Sumegi J (1993): X-linked lymphoproliferative disease. American Journal of Diseases of Children 147:1242–1245.

Seigneurin JM, Lavoue MF, Genoulaz O, Bornkamm GW, Lenoir GM (1987): Antibody response against the Epstein-Barr virus-coded nuclear antigen 2 (EBNA2) in different groups of individuals. International Journal of Cancer 40:349–353.

Sixbey JW, Shirley P, Sloas M, Raab-Traub N, Israele V (1991): A transformation-incompetent, nuclear antigen 2-deleted Epstein-Barr virus associated with replicative infection. Journal of Infectious Diseases 163:1008–1015.

Starr SE, Hansen-Flaschen J, Douglas SD, Perussia B (1990): Reappearance of natural-killer cell activity. New England Journal of Medicine 322:133 (letter).

Thomson SA, Khanna R, Gardner J, Burrows SR, Coupar B, Moss DJ, Suhrbier A (1995): Minimal epitopes expressed in a recombinant polyepitope protein are processed and presented to CD8+ cytotoxic T cells: Implications for vaccine design. Proceedings of the National Academy of Sciences USA 92:5845–5849.

Ubol S, Tucker PC, Griffin DE, Hardwick JM (1994): Neurovirulent strains of Alphavirus induce apoptosis in bcl-2-expressing cells: role of a single amino acid change in the E2 glycoprotein. Proceedings of the National Academy of Sciences USA 91:5202–5206.

Walling DM, Perkins AG, Webster-Cyriaque J, Resnick L, Raab-Traub N (1994): The Epstein-Barr virus EBNA-2 gene in oral hairy leukoplakia: Strain variation, genetic recombination, and transcriptional expression. Journal of Virology 68:7918–7926.